# Exposure to Indoor Background Radiation and Urinary Concentrations of 8-Hydroxydeoxyguanosine, a Marker of Oxidative DNA Damage

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We investigated whether exposure to indoor γ-radiation and radon might be associated with enough free radical formation to increase urinary concentrations of 8-hydroxydeoxyguanosine (8-OHdG), a sensitive marker of DNA damage, due to a hydroxyl radical attack at the C8 of guanine. Indoor radon and γ-radiation levels were measured in 32 dwellings for 6 months by solid-state nuclear track detectors and thermoluminescent dosimeters, respectively. Urine samples for 8-OHdG determinations were obtained from 63 healthy adult subjects living in the measured dwellings. An overall tendency toward increasing levels of 8-OHdG with increasing levels of radon and γ-radiation was seen in the females, presumably due to their estimated longer occupancy in the dwellings measured. Different models were considered for females, with the steepest slopes obtained for γ-radiation with a coefficient of 0.500 (log nmol/l of 8-OHdG for each unit increase of γ-radiation on a log scale) ($p<0.01$), and increasing to 0.632 ($p = 0.035$), but with larger variance, when radon was included in the model. In conclusion, there seems to be an effect of indoor radioactivity on the urinary excretion of 8-OHdG for females, who are estimated to have a higher occupancy in the dwellings measured than for males, for whom occupational and other agents may also influence 8-OHdG excretion. *Key words*: free radicals, γ-radiation, radon. *Environ Health Perspect* 107:213–215 (1999). [Online 2 February 1999] *http://ehpnet1.niehs.nih.gov/docs/1999/107p213-215sperati/abstract.html*

Whole-body γ-radiation causes leukemia and other cancers in humans (*1–3*). Ionizing radiation generates free radicals *in vivo*, including the highly reactive •OH (*4*). Oxidative damage to DNA may be a key component of the mechanism of action of different carcinogens, including γ-radiation (*5*). However, several pathways and complex interrelations seem to be involved in the causation of tumors.

Radon and its alpha-emitting progeny (hereafter referred to as radon) are among the few agents that have been established as carcinogenic to humans (*6*). The principal adverse effect arising from the inhalation of radon is lung cancer (*7*), but there are also possibly increased occurrences of other cancers in relation to elevated levels of radon (*8*). Health effects of radon seem to include chromosomal aberrations and mutations in lymphocytes (*6,9*), chromosomal instability in bone marrow cells (*10*), and free-radical formation (*7*).

The modified base 8-hydroxydeoxyguanosine (8-OHdG), an oxidative adduct form of deoxyguanosine, is considered a sensitive marker of the DNA damage due to a hydroxyl radical attack at the C8 of guanine (*11*). Such damage is usually successfully repaired by competent cells, but if unrepaired, the presence of 8-OHdG in DNA templates may cause the miscoded incorporation of nucleotides in the replicated strand (*12*), which may contribute to the development of cancer. Automated analytical methods have been developed to measure 8-OHdG in human cells (*13*) or urine (*14*). 8-OHdG measured in cell DNA represents a steady-state level (a balance between DNA damage and cellular repair), whereas the exact source of urinary excretion is not well known because the potentially involved repair pathway has not been identified. However, there is good evidence that urinary 8-OHdG is a marker of oxidative stress (*15*).

Several circumstances may affect 8-OHdG excretion, however. Thus, daily total intake of vitamin C has been significantly correlated with lower levels of 8-OHdG in urine (*15*). Some small-scale studies have indicated that 8-OHdG urinary levels may increase with physical exercise (*16*) and also among people with alcohol sensitivity (*17*), those with diabetes (*18*), and because of chemotherapy (*19–20*), as well as from occupational exposure to carcinogens (*21,22*). Here we report the results of a study undertaken to investigate whether exposure to indoor γ-radiation and radon in dwellings might be associated with increased urinary concentrations of 8-OHdG.

## Materials and Methods

The study was conducted in the Viterbo province (central Italy), where the soil has the potential for greater radiation emission than that in nearby areas (*23*). Furthermore, a building material created from local volcanic rocks (tuff) is widely used and also emits radon and thoron (*24*).

***Exposure assessment.*** Radon and γ-radiation levels were measured over a period of 6 months, starting between November 1993 and March 1994 (*23*). In each dwelling two detectors for radon and two for γ-radiation were placed in the main bedroom of the home. Radon was measured by solid-state nuclear track detectors (LR-115, Kodak Dosira, Lognes, France) and γ-radiation was measured using thermoluminescent dosimeters (GR200A, DML, Beijing, China). Results were expressed as the mean value of the readings of the two detectors for radon and the two for γ-radiation, respectively.

***Study population.*** We enrolled 63 healthy subjects who agreed to provide a urine sample. These subjects represented 32 dwellings. Information about their characteristics in terms of sex, age, smoking habits, and occupation was collected through questionnaires. Each subject was classified according to the degree of urbanization of the area of residence, and all 32 homes were classified according to the year of construction and the main building material. Finally, each subject was classified on the basis of his or her occupation as having mainly indoor or outdoor daytime activity.

***Urine collection, sample preparation, and chemical determination.*** After informed consent, a sample of at least 20 ml of untimed early morning urine was collected for each participant. All samples were collected and handled identically and the sample collection period extended from 19 July to 11 November 1994. Samples were subdivided into two 10-ml allocates and were stored at -20°C in conical tubes (Falcon 2070, Falcon, Lincoln Park, NJ) without any additives, then sent by air to Linköping, Sweden, in a box containing dry ice.

The pH adjustment and the processing sequence for extraction of 8-OHdG followed procedures described in detail by Lagorio et al. (*21*). Concentrations of 8-OHdG were determined by HPLC with electrochemical detection (*25*) and adjusted for density. Standard curves were linear up to at least 500 nmol/l, and the detection

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limit was 1.6 nmol/l. A quality control program was included in the analysis; three control samples (high, medium, and low levels) were taken at the beginning, middle, and end of each series. These control samples were prepared by addition of 8-OHdG to a 24-hr urine collection from a healthy individual. Because of this quality control program, there was no drift in the method. Previous investigations showed that the coefficient of variation within the series (calculated from analyses of control samples run at each working session) was 8–24%; between series the coefficient of variation was 8–23%, depending on the concentration level (*25*). The coefficient of variation within the series, calculated from analysis of duplicate samples, was 4.9–6.8% (*25*).

***Statistical analyses.*** One-way analysis of variance (ANOVA) was used to test the significance of differences in geometric mean concentrations of 8-OHdG by potentially predictive variables (age, smoking habit, occupation, period of construction and building material of the home, and quartiles of indoor radon and γ-radiation) for males and females separately. Males and females were evaluated separately because the ANOVA tests for interaction between sex and building material, radon, and γ-radiation were all statistically significant ($p<0.05$). A multiple linear regression analysis was then performed to study the effect of indoor radiation on the measured concentration of 8-OHdG. Because the measurements of exposure were not independent for all subjects (i.e., in several dwellings there was more than one subject), we used generalized estimating equations (GEE) to obtain the regression coefficients accounting for the dependence among observations from the same cluster (*26,27*). Diagnostic plots of standardized regression residuals were produced to verify the appropriateness of fitting a linear model to the data. The ANOVA was carried out using the SPSS/PC+ statistical package (*28*); we used the SPIDA software (*29*) for the GEE multiple linear regression analysis.

## Results

Table 1 shows the main characteristics of the study subjects and the geometric mean of urinary concentrations of 8-OHdG for males and females separately. Among males, the excretion of 8-OHdG was not affected by the variables of interest—including radon and γ-radiation. Among females, 8-OHdG was highest for women living in houses built with tuff and among those in the 3rd and 4th quartile of radon and γ-radiation exposure, respectively. A significant correlation was also observed between the log-transformation of both radon ($r = 0.315$; $p = 0.05$) and γ-radiation ($r = 0.405$; $p = 0.01$) and the log concentrations of urinary 8-OHdG in females.

**Table 1.** Geometric mean (GM) concentrations and geometric standard deviation (GSD) of urinary 8-hydroxydeoxyguanosine (8-OHdG) (nmol/l) among dwellers of the Viterbo (Italy) area by relevant characteristics of the subjects

| Characteristics | Males ($n = 24$), urinary 8-OHdG nmol/l | | | | Females ($n = 39$), urinary 8-OHdG nmol/l | | | |
|---|---|---|---|---|---|---|---|---|
| | *n* | GM | GSD | *p*-Value* | *n* | GM | GSD | *p*-Value* |
| Age | | | | | | | | |
| <30 | 8 | 24.8 | 1.5 | 0.79 | 9 | 16.1 | 1.7 | 0.87 |
| 30–49 | 6 | 21.5 | 1.1 | | 3 | 19.1 | 2.2 | |
| 50–69 | 10 | 23.1 | 1.6 | | 15 | 15.0 | 1.5 | |
| ≥70 | – | – | – | | 12 | 15.3 | 1.6 | |
| Smokers | | | | | | | | |
| No | 14 | 22.6 | 1.4 | 0.75 | 36 | 16.1 | 1.6 | 0.21 |
| Yes | 10 | 23.8 | 1.5 | | 3 | 11.2 | 1.2 | |
| Occupation | | | | | | | | |
| Retired/housewife | 3 | 22.0 | 1.1 | 0.92 | 27 | 15.5 | 1.6 | 0.82 |
| Farmer/blue collar | 11 | 23.1 | 1.5 | | 6 | 13.9 | 1.6 | |
| Clerk | 6 | 25.0 | 1.6 | | – | – | – | |
| Student/unemployed | 4 | 21.3 | 1.2 | | 4 | 14.0 | 1.2 | |
| Unknown | – | – | – | | 2 | – | – | |
| Building material | | | | | | | | |
| Concrete | 8 | 23.8 | 1.5 | 0.79 | 9 | 11.6 | 1.4 | 0.02 |
| Tuff | 16 | 22.9 | 1.4 | | 30 | 17.1 | 1.6 | |
| Radon (Bq/m$^3$) | | | | | | | | |
| 1st quartile (29–52) | 7 | 24.3 | 1.6 | 0.91 | 8 | 10.3 | 1.4 | 0.01 |
| 2nd quartile (53–72) | 7 | 24.3 | 1.7 | | 10 | 15.8 | 1.7 | |
| 3nd quartile (73–215) | 4 | 22.4 | 1.2 | | 11 | 20.7 | 1.5 | |
| 4th quartile (216–497) | 6 | 21.1 | 1.2 | | 10 | 16.0 | 1.5 | |
| Gamma (nGy/hr) | | | | | | | | |
| 1st quartile (145–220) | 6 | 23.8 | 1.7 | 0.17 | 9 | 10.6 | 1.4 | 0.003 |
| 2nd quartile (221–301) | 8 | 25.3 | 1.6 | | 9 | 18.2 | 1.6 | |
| 3nd quartile (302–407) | 5 | 22.9 | 1.1 | | 11 | 14.3 | 1.4 | |
| 4th quartile (408–772) | 5 | 19.5 | 1.2 | | 10 | 21.5 | 1.6 | |

*p-Value from analysis of variance.

**Table 2.** Estimated β-coefficients from the multiple regression analysis using generalized estimating equations[a]

| | Total women ($n = 39$) | | | Predominant daytime activity[b] | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Outdoors ($n = 10$) | | | Indoors ($n = 27$) | | |
| | β | SE (β) | *p*-Value | β | SE (β) | *p*-Value | β | SE (β) | *p*-Value |
| Radon | 0.162 | 0.103 | 0.12 | 0.140 | 0.129 | 0.28 | 0.200 | 0.112 | 0.074 |
| Gamma | 0.500 | 0.180 | <0.01 | 0.074 | 0.119 | 0.54 | 0.582 | 0.221 | 0.001 |

SE, standard error.

[a]Relation between the concentration of urinary 8-OHdG (nmol/l, log transform) and home levels of radon or γ-radiation (log), adjusted for age and smoking habit of the female subjects, according to their predominant daytime activity (indoors or outdoors).

[b]Two subjects who could not be classified according to this variable were not considered in this analysis.

In the GEE multiple linear regression analysis relating the concentration of urinary 8-OHdG to radon (Table 2) while controlling for a priori relevant variables (age group and smoking habits), we estimated an increase of 0.162 log nmol/l of 8-OHdG for each unit increase of radon on a log scale ($p = 0.12$). A separate model, where γ-radiation was included instead of radon, showed a steeper slope ($\beta = 0.500$) and a higher statistical significance for γ-radiation ($p = 0.006$) as compared to radon. In a model containing both radon and γ-radiation, there was no effect from radon, whereas γ-radiation reached statistical significance ($p = 0.035$) with a slight increase in its estimated coefficient ($\beta = 0.632$) and a larger standard error (0.300) due to collinearity problems. When data were analyzed separately for women according to their predominant daytime activity (outdoors or indoors) (Table 2), a statistically significant effect was observed for γ-radiation (both in the models with and without radon) only among women who spent most of their daytime indoors.

## Discussion

This study showed an association between both indoor radon and γ-radiation levels with concentrations of urinary 8-OHdG among females. The multivariate analysis indicated that the role of γ-radiation was predominant as compared to that of radon.

Males had consistently higher concentrations of 8-OHdG, as compared to females, for all levels of all variables of interest. This would confirm previous observations that urinary excretion of 8-OHdG is influenced by constitutional factors, such as sex and body mass index, in addition to environmental exposures such as smoking and chemo- or radiotherapy (*13,19,20,21*). Only 3 of 24 of the male subjects in the study had predominantly indoor daytime activities (i.e., were retired); therefore, it might be argued that exposures outside the home could have had the most substantial influence on the urinary concentrations of 8-OHdG. This is corroborated by the fact that only females showed differences in 8-OHdG urinary concentrations in relation to the building material of the home. Measurements of 8-OHdG among males could have been influenced by occupational exposures because chemicals such as pesticides are frequently used in the mainly rural area where the study was undertaken. Furthermore, because exposures to radon and γ-radiation were not measured at workplaces, females (27 of 37) had on the whole a better assessment for the specific exposures of interest in our study. It is noteworthy that women with mainly indoor daytime activity had much steeper slopes both for radon and γ-radiation than women with mainly outdoor daytime activity (Table 2).

In our study population, cigarette smoking did not appear to affect urinary 8-OHdG levels among males or females; this is in accordance with occupational studies (*21,22*), but in contrast with another study on healthy people (*30*).

This study has many limitations due to its small size and lack of dietary assessment, body mass index, and a detailed time–activity pattern. Furthermore, the correlation between the higher levels of 8-OHdG and indoor radiation levels appears to depend on the highest exposure levels. However, our observations suggest an effect of indoor radioactivity on the urinary excretion of 8-OHdG in the population subgroup for which the exposure assessment is most appropriate (i.e., for females—in particular for those with at-home daily activities). Our analysis indicates that this effect might be due to γ-radiation and not to radon; the effect of radon disappears when it is included in a model simultaneously with γ-radiation. These findings are in agreement with experimental evidence of oxidative mutagens produced by radiation (*4*). A reason for the stronger effect of γ-radiation could be that there is a whole-body exposure to γ-radiation, whereas the radon progeny exerts a localized irradiation of the bronchii only. It is possible that the highly energetic alpha-particles emitted in the decay of radon and its progeny would cause more severe and destructive damage to DNA than the less energetic γ-radiation. Hence, the free radicals involved in the 8-OHdG formation may reflect exposure to the latter type of radiation.

Further studies are warranted to confirm or refute our results, possibly by measuring 8-OHdG in lymphocyte DNA to further evaluate whether the steady-state level of DNA damage is elevated as a result of γ-radiation or radon exposure. Future studies might be based on better exposure assessment, possibly including (when applicable) measurements of radon and γ-radiation at the workplace, as well as measurement of other factors such as diet and various occupational exposures as potentially affecting urinary excretion of 8-OHdG. Although chance phenomena can never be excluded, it is unlikely that factors of this kind could have had any greater influence by confounding the results of this study. Such factors are unlikely to have been associated with measured exposure to indoor radioactivity, which is a necessary requirement for the exertion of any confounding effect. However, some occupational exposures among the males may have caused a blurring effect by increasing the background levels of 8-OHdG so as to make the effect of indoor radiation invisible among subjects who spent only limited time in the measured dwellings.

## References and Notes


1. Linet MS. The Leukemias: Epidemiologic Aspects. New York:Oxford University Press, 1985;123–152.
2. Boice JD Jr, Land CE. Ionizing radiation. In: Cancer Epidemiology and Prevention (Schottenfeld D, Fraumeni JF Jr, eds). Philadelphia:WB Saunders Co, 1982;231–253.
3. Boice JD Jr, Fraumeni JF Jr, eds. Radiation Carcinogenesis: Epidemiology and Biological Significance, Vol 26: Progress in Cancer Research and Therapy. New York:Raven Press, 1984.
4. Von Sonntag C. The Chemical Basis of Radiation Biology. London:Taylor and Francis, 1987.
5. Harris CC. Tumour suppressor genes, multistage carcinogenesis and molecular epidemiology. In: Mechanisms of Carcinogenesis in Risk Identification (Vainio H, Magee P, McGregor DB, McMichael AJ, eds). IARC Scientific Publications No 116. Lyon:International Agency for Research on Cancer, 1992;67–85.
6. IARC. Radon. In: Monographs on the Evaluation of Carcinogenic Risks to Humans, Vol 43: Man-Made Mineral Fibres and Radon. Lyon:International Agency for Research on Cancer, 1988;173–259.
7. Axelson O. Radon. In: Environmental Epidemiology: Exposure and Disease (Bertollini R, Lebowitz MD, Saracci R, Savitz DA, eds). Boca Raton, FL:Lewis Publishers, 1996;19–35.
8. Henshaw DL, Eatough JP, Richardson RB. Radon: a causative factor in the induction of myeloid leukemia and other cancers in adults and children? Lancet 335:1008–1012 (1990).
9. Bridges BA, Cole J, Arlett CF, Green MH, Waugh AP, Beare D, Henshaw DL, Last RD. Possible association between mutant frequency in peripheral lymphocytes and domestic radon concentrations. Lancet 337:1187–1189 (1991).
10. Kadhim MA, Lorimore SA, Hepburn MD, Goodhead DT, Buckle VJ, Wright EG. I-particle-induced chromosomal instability in human bone marrow cells. Lancet 344:987–988 (1994).
11. Kasai H, Crain PF, Kuchino Y, Nishimura S, Ootsuyama A, Tanooka H. Formation of 8-hydroxyguanosine moiety in cellular DNA by agents producing oxygen radicals and evidence for its repair. Carcinogenesis 7:1849–1851 (1989).
12. Kuchino Y, Mori F, Kasai H, Inoue H, Iwai S, Miura K, Ohtsuka E, Nishimura S. Misreading of DNA templates containing 8-deoxyhydroxyguanosine at the modified base and at adjacent residues. Nature 327:77–79 (1987).
13. Nakajima M, Takeuchi T, Morimoto K. Determination of 8-hydroxydeoxyguanosine in human cells under oxygen-free conditions. Carcinogenesis 17:787–791 (1996).
14. Tagesson C, Källberg M, Leandersson P. Determination of urinary 8-hydroxydeoxyguanosine by couple-column HPLC with electrochemical detection: a noninvasive assay for *in vivo* oxidative DNA damage in humans. Toxicol Methods 1:242–251 (1992).
15. Whitherell HL, Hiatt RA, Replogle M, Parsonnet J. *Helicobacter pylori* infection and urinary excretion of 8-hydroxy-2-deoxyguanosine, an oxidative DNA adduct. Cancer Epidemiol Biomarkers Prev 7:91–96 (1998).
16. Inoue T, Mu Z, Sumikawa K, Adachi K, Okochi T. Effect of physical exercise on the content of 8-hydroxydeoxyguanosine in nuclear DNA prepared from human lymphocytes. Jpn J Cancer Res 84:720–725 (1993).
17. Nakajima M, Takeuchi T, Takeshita T, Morimoto K. 8-Hydroxydeoxyguanosine in human leukocyte DNA and daily health practice factors: effects of individual alcohol sensitivity. Environ Health Perspect 104:1336–1338 (1996).
18. Dandona P, Thusu K, Cook S, Snyder B, Makowski J, Armstrong D, Nicotera T. Oxidative damage to DNA in diabetes mellitus. Lancet 347:444–445 (1996).
19. Bergtold DS, Berg CD, Simic MC. Urinary biomarkers in radiation therapy of cancer. In: Antioxidants in Therapy and Preventive Medicine (Emerit I, Packer L, Auclair C, eds). New York:Plenum Press, 1990;311–316.
20. Shigenaga MK, Ames BN. Assays for 8-hydroxy-2-deoxyguanosine: a biomarker of *in vivo* oxidative DNA damage. Free Radical Biol Med 10:211–216 (1991).
21. Lagorio S, Tagesson C, Forastiere F, Iavarone I, Axelson O, Carere A. Exposure to benzene and urinary concentrations of 8-hydroxydeoxyguanosine, a biological marker of oxidative damage to DNA. Occup Environ Med 51:739–743 (1994).
22. Tagesson C, Chabiuk D, Axelson O, Baranski B, Palus J, Wyzynska K. Increased urinary excretion of the oxidative DNA adduct 8-hydroxydeoxyguanosine, as a possible early indicator of occupational cancer hazards in the asbestos, rubber, and azo-dye industry. Pol J Occup Med Environ Health 6:357–368 (1993).
23. Forastiere F, Sperati A, Cherubini G, Miceli M, Biggeri A, Axelson O. Adult myeloid leukemia, geology, and domestic exposure to radon and γ radiation: a case control study in central Italy. Occup Environ Med 55:106–110 (1998).
24. Sciocchetti G, Clemente FG, Ingrao G, Sacco F. Results of a survey on radioactivity of building materials in Italy. Health Phys 45:385–388 (1983).
25. Tagesson C, Källberg M, Klintenberg C, Starkhammar H. Determination of urinary 8-hydroxydeoxyguanosine by automated coupled-column high performance liquid chromatography: a powerful technique for assaying *in vivo* oxidative DNA damage in cancer patients. Eur J Cancer 31A:934–940 (1995).
26. Liang KY, Zeger SL. Longitudinal data analysis using generalized linear models. Biometrika 73:13–22 (1986).
27. Liang KY, Zeger SL. Regression analysis for correlated data. Annu Rev Publ Health 14:43–68 (1993).
28. Norusis MJ, SPSS Inc. SPSS/PC+ V3.0 Update Manual. Chicago, IL:SPSS Inc.,1988.
29. Gebski V, Leung O, McNeil D, Lunn D. SPIDA User's Manual. Macquarie University, New South Wales, Australia:Statistical Computing Lab, 1992.
30. Loft S, Vistisen K, Ewertz M, Tjonneland A, Overvad K, Poulsen HE. Oxidative DNA damage estimated by 8-hydroxydeoxyguanosine excretion in humans: influence of smoking, gender, and body mass index. Carcinogenesis 13:2241–2247 (1992).